

ASSOCIATION BETWEEN PLASMA HOMOCYSTEINE CONCENTRATIONS AND EXTRACRANIAL CAROTID-ARTERY STENOSIS

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Abstract Background. Epidemiologic studies have identified hyperhomocysteinemia as a possible risk factor for atherosclerosis. We determined the risk of carotid-artery atherosclerosis in relation to both plasma homocysteine concentrations and nutritional determinants of hyperhomocysteinemia.

Methods. We performed a cross-sectional study of 1041 elderly subjects (418 men and 623 women; age range, 67 to 96 years) from the Framingham Heart Study. We examined the relation between the maximal degree of stenosis of the extracranial carotid arteries (as assessed by ultrasonography) and plasma homocysteine concentrations, as well as plasma concentrations and intakes of vitamins involved in homocysteine metabolism, including folate, vitamin B₁₂, and vitamin B₆. The subjects were classified into two categories according to the findings in the more diseased of the two carotid vessels: stenosis of 0 to 24 percent and stenosis of 25 to 100 percent.

Results. The prevalence of carotid stenosis of ≥25 percent was 43 percent in the men and 34 percent in the women. The odds ratio for stenosis of ≥25 percent was 2.0 (95 percent confidence interval, 1.4 to 2.9) for subjects with the highest plasma homocysteine concentrations (≥14.4 μmol per liter) as compared with those with the lowest concentrations (≤9.1 μmol per liter), after adjustment for sex, age, plasma high-density lipoprotein cholesterol concentration, systolic blood pressure, and smoking status (P<0.001 for trend). Plasma concentrations of folate and pyridoxal-5'-phosphate (the coenzyme form of vitamin B₆) and the level of folate intake were inversely associated with carotid-artery stenosis after adjustment for age, sex, and other risk factors.

Conclusions. High plasma homocysteine concentrations and low concentrations of folate and vitamin B₆, through their role in homocysteine metabolism, are associated with an increased risk of extracranial carotid-artery stenosis in the elderly. (N Engl J Med 1995;332:286-91.)

McCULLY made the initial observation linking plasma homocysteine concentrations and arteriosclerotic vascular disease more than 25 years ago.¹ He reported that an infant with homocystinuria who died as a result of a rare inborn error of cobalamin metabolism had widespread, severe arteriosclerosis analogous to the lesions seen in cases of homocystinuria caused by cystathionine β-synthase deficiency. Because an elevated plasma homocysteine concentration was the only metabolic abnormality common to these two hereditary enzyme disorders, McCully proposed that hyperhomocysteinemia resulted in arteriosclerotic disease.

The association between the plasma homocysteine concentration and atherosclerosis has more recently become the subject of a number of clinical studies, which have consistently linked moderate hyperhomocysteinemia to symptomatic peripheral vascular, cerebrovascular, and coronary heart disease.^{2,3} Pooled results from retrospective studies indicate that fasting homocysteine concentrations in patients with vascular disease are on average 31 percent higher than in normal subjects, and an abnormal homocysteine concen-

tration after an oral methionine challenge is 12 times more prevalent in patients than in normal subjects.² A recent prospective study of middle-aged male physicians in the United States indicated that plasma homocysteine concentrations only 1.7 μmol per liter, or 12 percent, above the upper limit of normal were associated with a 3.4-fold increase in the risk of acute myocardial infarction.⁴

The advent of noninvasive ultrasound imaging methods has made it possible to study risk factors for arteriosclerosis of the extracranial carotid artery in large populations.⁵⁻¹³ Ultrasonographic findings of carotid-artery arteriosclerosis have proved to be a useful predictor of systemic atherothrombosis,^{11,14-21} especially coronary heart disease^{11,15-18,20,21} and cerebrovascular disease.^{11,19}

Carotid-artery ultrasonography has been used to examine the association between plasma homocysteine concentrations and arteriosclerosis. Obligate heterozygotes for cystathionine β-synthase deficiency were shown to have a greater prevalence of carotid-artery arteriosclerosis than normal subjects,²² and elevated plasma homocysteine concentrations after methionine loading were associated with asymptomatic carotid-artery arteriosclerosis in a combined sample of obligate heterozygotes for cystathionine β-synthase deficiency and a similar number of control subjects.²³ Another study demonstrated that persons with carotid-artery walls whose thickness exceeded the 90th percentile for the study cohort had significantly higher fasting plasma homocysteine concentrations than persons with carotid-artery walls whose thickness was below the 75th percentile.²⁴

We examined the relation between carotid-artery stenosis, as assessed by ultrasonography, and plasma concentrations of homocysteine in the Framingham

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Heart Study cohort. We also studied concentrations of folate, vitamin, B₁₂, and pyridoxal-5'-phosphate (the coenzyme form of vitamin B₆), which are nutritional determinants of hyperhomocysteinemia. We had previously demonstrated that hyperhomocysteinemia was prevalent in this cohort and was associated in most cases with inadequate vitamin concentrations.²⁵

METHODS

Subjects

The participants were members of the original Framingham Heart Study cohort, a population-based sample of 5209 men and women originally examined between 1948 and 1952²⁶ and then followed prospectively every two years to assess the occurrence of vascular disease. The present study was based on 1401 survivors of the original cohort who participated in the 20th biennial examination (1989 to 1990). Plasma homocysteine concentrations and the results of ultrasonographic measurements of carotid-artery stenosis were available for 1041 subjects (418 men and 623 women) who were 67 to 96 years old at the time of data collection. Informed consent was obtained from all participants. The protocols for this study were approved by the Human Investigations Review Committee at New England Medical Center and by the Institutional Review Board for Human Research at Boston University Medical Center.

Measurement of Carotid-Artery Stenosis

At the 20th biennial examination, participants underwent a Doppler examination of the carotid arteries with a high-resolution, real-time scanner equipped with a 7.5-MHz imaging transducer, a 4-MHz pulse-wave Doppler transducer, and a 4-MHz continuous-wave transducer. The left and right carotid bifurcations were each studied in three projections including the distal 1 cm of the common carotid artery, the carotid bulb, and the proximal 1 cm of the internal carotid artery. Frozen images were captured on a page printer. Both the frozen image and a short segment of real-time scanning (to demonstrate motion) were videotaped for later interpretation. Continuous-wave Doppler recordings of the external carotid artery and both pulsed-wave and continuous-wave recordings of the carotid bifurcation exclusive of the external carotid artery were obtained at the site of maximal flow disturbance.

Studies were analyzed in a blinded fashion by a single reader. The thickness of plaques at the near and far wall exclusive of the external carotid artery was measured at the site of maximal disease in each view with hand-held calipers. The total thickness was determined by averaging the sum of the near- and far-wall measurements in each of the three projections imaged. Residual lumen was measured just distal to the site of any wall abnormality. Peak systolic velocities and frequencies were recorded from sites of maximal flow disturbance. Both Doppler spectral criteria and assessment of gray-scale images were used to estimate vascular stenosis. The right and left carotid arteries were assessed separately. We classified subjects into two categories on the basis of the maximal percentage of stenosis of the more diseased of the two arteries: 0 to 24 percent or 25 to 100 percent. The use of this cutoff point is based on three considerations. First, the maximal percentage of stenosis is measured as an ordinal variable with grouping of values at discrete intervals of 5 (e.g., 0, 5, 10, and 15 percent). Second, O'Leary et al.¹¹ demonstrated a significantly increased prevalence of both stroke and coronary heart disease among persons with maximal stenosis of the carotid arteries of more than 25 percent. Third, the results were not specific to the 25 percent cutoff, since similar results were obtained when values of 40 percent and 50 percent were used, but the statistical power was reduced because of the smaller numbers of subjects with maximal percentages of stenosis above these cutoff points.

Biochemical Determinations

Blood was drawn from nonfasting subjects, and plasma total cholesterol and high-density lipoprotein (HDL) cholesterol were measured in the Framingham Heart Study laboratory with enzymatic methods.^{27,28} Low-density lipoprotein cholesterol was not measured because the blood samples were taken from nonfasting subjects. Plas-

ma samples stored at -80°C were used for the measurement of total homocysteine according to the method of Araki and Sako.²⁹ Plasma folate was measured by a microbial assay with a 96-well plate and manganese supplementation as described by Tamura et al.³⁰ Vitamin B₁₂ was measured with a radioassay kit (Magic, Ciba-Corning, Medfield, Mass.), and pyridoxal-5'-phosphate was measured by the tyrosine decarboxylase method as described by Camp et al.³¹ Because of insufficient plasma, vitamin concentrations were not measured in all subjects.

Assessment of Nutrient Intake

Members of the cohort were mailed a semiquantitative food-frequency questionnaire³² when they were scheduled for their 20th biennial examination. The subjects returned the completed questionnaire at the time of their examination. This questionnaire permits the estimation of nutrient intake for ranking or categorizing subjects, but it does not provide precise quantitative measures of nutrient intake. Intakes of folate and vitamin B₆ estimated with the use of this questionnaire in other population samples corresponded well with plasma folate and pyridoxal-5'-phosphate concentrations and with folate and vitamin B₆ intakes measured with the use of dietary records, whereas vitamin B₁₂ intakes estimated with this questionnaire were only moderately well correlated with plasma vitamin B₁₂ concentrations.³²⁻³⁴ In the Framingham cohort, intakes of folate and vitamin B₆ estimated on the basis of this questionnaire also correlated well with plasma folate and pyridoxal-5'-phosphate concentrations, respectively, but vitamin B₁₂ intake was not correlated with plasma vitamin B₁₂ concentrations.²⁵ The weaker correlation for vitamin B₁₂ in this elderly cohort might be explained by diminished absorption of vitamin B₁₂ from foods associated with the age-related increase in the incidence of atrophic gastritis.³⁵

Statistical Analysis

To describe graphically the relation of plasma homocysteine concentrations to stenosis, we classified men and women according to their homocysteine concentrations (≤ 9.1 , 9.2 to 11.3, 11.4 to 14.3, and ≥ 14.4 μmol per liter). Within each quartile, we computed the prevalence of carotid-artery stenosis of ≥ 25 percent and plotted the prevalence estimates at the sex-specific median homocysteine concentration for that quartile.

To adjust for other risk factors for carotid-artery stenosis, logistic regression was used with stenosis of ≥ 25 percent as the dependent variable. Homocysteine quartiles were modeled with indicator variables to represent the three highest quartiles, and the relative risk of stenosis of ≥ 25 percent for each quartile as compared with the risk for the lowest quartile was estimated as the odds ratio derived as the antilogarithm of the logistic-regression coefficients. To examine the association between the nutritional determinants of plasma homocysteine and stenosis, we also divided subjects into four groups according to their concentrations of each vitamin measured and represented them in the regression models as indicator variables, using the highest quartile as the reference category to estimate the relative risk of lower nutrient levels.

Because of missing data on plasma vitamin concentrations and nutrient intake, we divided the subjects into five subgroups. We examined the relation between extracranial carotid-artery stenosis and plasma homocysteine concentrations among all subjects who had had carotid-artery ultrasonography and plasma homocysteine measurements ($n = 1041$). We next considered the relations between stenosis and plasma concentrations of folate ($n = 1027$), vitamin B₁₂ ($n = 881$), pyridoxal-5'-phosphate ($n = 967$), and all vitamins simultaneously ($n = 812$). Finally, we considered the relations between stenosis and nutrient intake ($n = 822$). All models that examined the relation of plasma vitamin concentrations or vitamin intake to carotid-artery stenosis were considered with and without homocysteine so that we could determine whether any associations between stenosis and the nutrients might be mediated by the plasma homocysteine concentration. A likelihood-ratio test statistic was calculated for the models that included the variable of plasma homocysteine plus the other plasma nutrients to determine the independent contribution of the plasma nutrients to stenosis with homocysteine in the model.

Initial analyses tested for a difference between the trend in the prevalence for women and for men by including an interaction term

between sex and homocysteine concentration in the regression model. Because the results did not reach statistical significance (P for interaction = 0.07), we present only the combined, sex-adjusted results. In addition to sex, all analyses were adjusted for age or age plus the ratio of total cholesterol to HDL cholesterol, smoking status, and systolic blood pressure. Analyses involving nutrient intake were also adjusted for energy intake. Tests for the trend of the odds ratio for stenosis across quartiles of homocysteine and vitamin concentrations were based on logistic regression of ordinal variables, with four levels to model exposure quartiles. All analyses were performed with SAS statistical software.³⁶ Unless otherwise noted, a two-sided P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Table 1 lists the characteristics of the study subjects. Forty-three percent of the men and 34 percent of the women had extracranial carotid-artery stenosis of ≥ 25 percent. The mean plasma homocysteine concentrations were $13.0 \mu\text{mol}$ per liter (range, 3.5 to 66.9) in the men and $12.5 \mu\text{mol}$ per liter (range, 3.5 to 64.6) in the women. Figure 1 shows the age-adjusted prevalence of stenosis across quartiles of plasma homocysteine concentrations. In the men, the prevalence of stenosis of ≥ 25 percent was 27 percent (95 percent confidence interval, 17 to 38 percent) in the lowest homocysteine quartile and 58 percent (95 percent confidence interval, 49 to 67 percent) in the highest quartile ($P < 0.001$ for trend). The relation in the women was not as strong as that in the men: the prevalence of stenosis of ≥ 25 percent ranged from 31 percent (95 percent confidence interval, 24 to 38 percent) to 39 percent (95 percent confidence interval, 31 to 47 percent) across homocysteine quartiles ($P = 0.03$ for trend). For men the risk of stenosis appeared to increase in the second homocysteine quartile (9.2 to $11.3 \mu\text{mol}$ per liter), but it did not appear to increase for women until the third quartile (11.4 to $14.3 \mu\text{mol}$ per liter). Although the prevalence of stenosis appeared somewhat greater among men than women in the upper quartiles, a test of interaction between sex and homocysteine

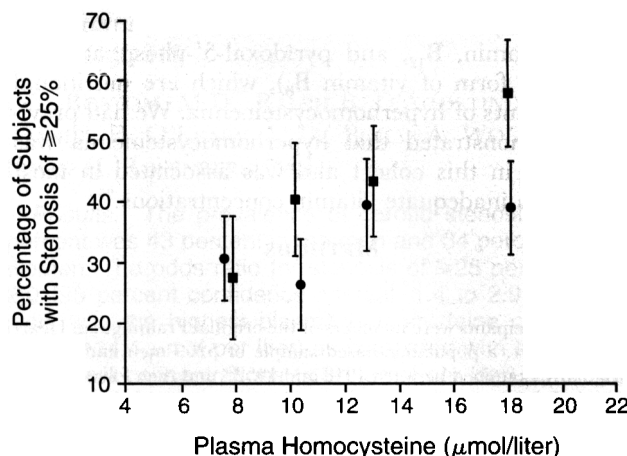


Figure 1. Age-Adjusted Prevalence of Maximal Extracranial Carotid-Artery Stenosis of ≥ 25 Percent in Men (■) and Women (●), According to the Quartile of Plasma Homocysteine Concentration.

The bars indicate the 95 percent confidence intervals. The quartiles of homocysteine concentrations were ≤ 9.1 , 9.2 to 11.3 , 11.4 to 14.3 , and $\geq 14.4 \mu\text{mol}$ per liter. The prevalence is plotted at the sex-specific median concentration for each quartile. (Test for linear trend, $P < 0.001$ for men and $P = 0.03$ for women.)

concentration indicated that the trends were not significantly different for men and women ($P = 0.07$).

The age- and sex-adjusted odds ratios for men and women combined were significantly increased in the third (odds ratio, 1.6; 95 percent confidence interval, 1.1 to 2.4) and fourth (odds ratio, 2.1; 95 percent confidence interval, 1.5 to 3.0) quartiles of homocysteine concentration (Table 2). Adjustment for other risk factors had little effect on the odds ratios. Likewise, the inclusion in the regression models of plasma folate, vitamin B_{12} , or pyridoxal-5'-phosphate concentrations, alone or combined, did not substantially alter the relation between stenosis and homocysteine concentrations (Table 2).

The associations between carotid-artery stenosis and the plasma vitamin concentrations are shown in Table 3. The prevalence of stenosis of ≥ 25 percent was inversely associated with both folate concentrations ($P < 0.001$ for trend) and pyridoxal-5'-phosphate concentrations ($P = 0.03$ for trend) after adjustment for age, sex, and other risk factors. The odds ratio for stenosis of ≥ 25 percent was 1.9 (95 percent confidence interval, 1.3 to 2.7) in the lowest folate quartile and 1.6 (95 percent confidence interval, 1.1 to 2.4) in the lowest pyridoxal-5'-phosphate quartile. Plasma vitamin B_{12} concentrations were weakly associated with stenosis ($P = 0.11$ for trend). The odds ratio for stenosis of ≥ 25 percent was 1.4 (95 percent confidence interval, 0.9 to 2.1) in the lowest vitamin B_{12} quartile as compared with the highest quartile. Adjustment for the homocysteine concentration diminished the strength of the plasma vitamin associations, but the prevalence of stenosis of ≥ 25 percent among subjects in the lowest plasma folate quartile remained elevated (odds ratio, 1.5; 95 percent confidence interval, 1.0 to 2.3).

The associations between carotid-artery stenosis and

Table 1. Characteristics of the Study Subjects.

CHARACTERISTIC*	MEN (N = 418)	WOMEN (N = 623)
	mean \pm SD	
Age (yr)	75 \pm 5	76 \pm 5
Stenosis of ≥ 25 % (%)	43 \pm 50	34 \pm 47
Homocysteine ($\mu\text{mol/liter}$)	13.0 \pm 5.5	12.5 \pm 6.0
Systolic blood pressure (mm Hg)	146 \pm 22	147 \pm 23
Plasma total cholesterol (mg/dl)	204 \pm 38	223 \pm 39
Plasma HDL cholesterol (mg/dl)	41 \pm 12	53 \pm 16
Total:HDL cholesterol ratio	5.4 \pm 1.8	4.6 \pm 1.6
Current smoker (%)	9.3 \pm 29.1	10.9 \pm 31.2
Plasma folate ($\mu\text{g/liter}$)	6.0 \pm 7.5	7.0 \pm 7.3
Plasma vitamin B_{12} (ng/liter)	41 \pm 12	474 \pm 231
Plasma pyridoxal-5'-phosphate (nmol/liter)	77 \pm 92	84 \pm 98
Folate intake ($\mu\text{g/day}$)	363 \pm 195	395 \pm 246
Vitamin B_{12} intake ($\mu\text{g/day}$)	10.8 \pm 28.9	9.2 \pm 8.7
Vitamin B_6 intake (mg/day)	5.0 \pm 16.3	4.6 \pm 14.3

*To convert values for cholesterol to millimoles per liter, multiply by 0.02586; to convert values for plasma folate to nanomoles per liter, multiply by 2.266; and to convert values for plasma vitamin B_{12} to picomoles per liter, multiply by 0.7378.

Table 2. Odds Ratio for Maximal Extracranial Carotid-Artery Stenosis of ≥ 25 Percent after Adjustment for the Plasma Concentrations of Various Vitamins, According to the Quartile of Plasma Homocysteine Concentration.

VITAMIN ADJUSTED FOR*	HOMOCYSTEINE QUANTILE ($\mu\text{mol/LITER}$)	NO. OF SUBJECTS	ADJUSTED FOR AGE AND SEX			ADJUSTED FOR MULTIPLE RISK FACTORS†		
			ODDS RATIO	95% CI‡	P VALUE	ODDS RATIO	95% CI‡	P VALUE
None	≤ 9.1	1041	1.0			1.0		
	9.2–11.3		1.1	0.8–1.6	0.60	1.1	0.8–1.6	0.58
	11.4–14.3		1.6	1.1–2.4	0.009	1.6	1.1–2.3	0.02
	≥ 14.4		2.1	1.5–3.0	<0.001§	2.0	1.4–2.9	<0.001§
Folate	≤ 9.1	1027	1.0			1.0		
	9.2–11.3		1.1	0.7–1.6	0.70	1.1	0.7–1.6	0.70
	11.4–14.3		1.5	1.0–2.2	0.04	1.4	1.0–2.1	0.06
	≥ 14.4		1.8	1.2–2.7	0.004	1.7	1.1–2.5	0.01
Vitamin B ₁₂	≤ 9.1	881	1.0			1.0		
	9.2–11.3		1.0	0.7–1.6	0.84	1.1	0.7–1.6	0.76
	11.4–14.3		1.6	1.0–2.3	0.04	1.5	1.0–2.3	0.05
	≥ 14.4		1.9	1.3–2.9	0.002	1.8	1.2–2.8	0.004
Pyridoxal-5'-phosphate	≤ 9.1	967	1.0			1.0		
	9.2–11.3		1.0	0.7–1.5	0.87	1.0	0.7–1.5	0.86
	11.4–14.3		1.5	1.0–2.2	0.06	1.4	1.0–2.1	0.08
	≥ 14.4		1.9	1.3–2.8	0.001	1.8	1.2–2.7	0.003
All vitamins	≤ 9.1	812	1.0			1.0		
	9.2–11.3		1.0	0.6–1.5	0.85	1.0	0.6–1.5	0.90
	11.4–14.3		1.4	0.9–2.2	0.13	1.4	0.9–2.2	0.14
	≥ 14.4		1.8	1.1–2.8	0.01	1.8	1.2–2.8	0.004

*The P values for the likelihood-ratio test statistic of the added contribution of the plasma nutrient or nutrients to the multivariate-adjusted homocysteine model are as follows: $P < 0.28$ for the addition of folate, $P < 0.57$ for the addition of vitamin B₁₂, $P < 0.29$ for the addition of pyridoxal-5'-phosphate, and $P < 0.79$ for the addition of all nutrients.

†Adjusted for sex, age, total:HDL cholesterol ratio, smoking status, and systolic blood pressure.

‡CI denotes confidence interval.

§For trend.

Table 3. Odds Ratios for Maximal Extracranial Carotid-Artery Stenosis of ≥ 25 Percent, According to the Quartile of Plasma Vitamin Concentrations.

VITAMIN*	NO. OF SUBJECTS	ADJUSTED FOR MULTIPLE RISK FACTORS†			ADJUSTED FOR MULTIPLE RISK FACTORS PLUS HOMOCYSTEINE CONCENTRATION		
		ODDS RATIO	95% CI‡	P VALUE	ODDS RATIO	95% CI‡	P VALUE
Folate ($\mu\text{g/LITER}$)	1027	1.9	1.3–2.7	0.001	1.5	1.0–2.3	0.04
		1.4	1.0–2.0	0.08	1.3	0.9–1.9	0.24
		1.2	0.8–1.8	0.28	1.2	0.8–1.8	0.35
		1.0			1.0		
Vitamin B ₁₂ (ng/LITER)	881			<0.001§			0.05§
		1.4	0.9–2.1	0.11	1.2	0.8–1.8	0.41
		1.4	0.9–2.0	0.14	1.2	0.8–1.8	0.36
		1.3	0.9–2.0	0.16	1.3	0.9–1.9	0.24
Pyridoxal-5'-phosphate (nmol/LITER)	967	1.0			1.0		
				0.11§			0.47§
		1.6	1.1–2.4	0.02	1.3	0.9–2.0	0.15
		1.1	0.7–1.6	0.67	1.0	0.6–1.4	0.80
		1.2	0.8–1.7	0.48	1.1	0.7–1.6	0.71
		1.0			1.0		

*To convert values for folate to nanomoles per liter, multiply by 2.266, and to convert values for vitamin B₁₂ to picomoles per liter, multiply by 0.7378.

†Adjusted for sex, age, total:HDL cholesterol ratio, smoking status, and systolic blood pressure.

‡CI denotes confidence interval.

§For trend.

vitamin intake are shown in Table 4. Although the trend was not as striking as that for plasma folate concentration, the data suggest that the prevalence of stenosis is higher among subjects with folate intakes below the highest quartile ($<475 \mu\text{g}$ per day) ($P = 0.04$ for trend). Like the association of carotid-artery stenosis with plasma vitamin concentrations, the observed association between folate intake and stenosis was substantially reduced but not entirely eliminated by adjustment for the homocysteine concentration. Neither vitamin B₁₂ intake nor vitamin B₆ intake was related to the prevalence of stenosis.

DISCUSSION

We found that plasma homocysteine concentrations are associated with extracranial carotid-artery stenosis in a population-based cohort of elderly people. We divided the cohort into two groups according to the extent of extracranial carotid-artery stenosis: 0 to 24 percent and 25 to 100 percent. Apart from statistical considerations described in the Methods section, we determined this cutoff point on the basis of evidence that a moderate degree of stenosis is associated with an increased risk of vascular events. O'Leary et al.¹¹ demonstrated a significantly increased prevalence of both stroke and coronary heart disease among persons with more than 25 percent stenosis of the carotid arteries. There is also evidence that a finding of moderate carotid-artery stenosis predicts incident disease. In a preliminary analysis of middle-aged men in eastern Finland, Salonen and Salonen¹⁶ found that the presence of carotid-artery stenosis of ≥ 20 percent on B-mode ultrasonography increased the estimated relative risk of incident myocardial infarction 6.7-fold. Using Doppler ultrasound criteria only, Aronow and colleagues have shown that more severe carotid-artery stenosis (≥ 40 percent) predicted both incident coronary heart disease¹⁷ and cerebrovascular events¹⁹ in unselected male and female residents of a long-term health care facility.

We demonstrated that the association between plasma homocysteine

Table 4. Odds Ratios for Maximal Extracranial Carotid-Artery Stenosis of ≥ 25 Percent in 822 Subjects, According to the Quartile of Vitamin Intake.

VITAMIN	ADJUSTED FOR MULTIPLE RISK FACTORS*			ADJUSTED FOR MULTIPLE RISK FACTORS PLUS HOMOCYSTEINE CONCENTRATION		
	ODDS RATIO	95% CI†	P VALUE	ODDS RATIO	95% CI†	P VALUE
Folate ($\mu\text{g/day}$)						
<230	1.5	1.0–2.4	0.07	1.3	0.8–2.0	0.35
230–326	1.8	1.2–2.8	0.006	1.5	1.0–2.3	0.06
327–474	1.5	1.0–2.2	0.08	1.3	0.9–2.1	0.19
≥ 475	1.0			1.0		
			0.04‡			0.28‡
Vitamin B ₁₂ ($\mu\text{g/day}$)						
<3.82	1.3	0.8–2.0	0.24	1.2	0.8–1.9	0.48
3.83–6.86	1.2	0.8–1.8	0.45	1.1	0.7–1.7	0.69
6.87–12.77	1.0	0.7–1.5	0.95	1.0	0.7–1.5	0.98
≥ 12.78	1.0			1.0		
			0.18‡			0.48‡
Vitamin B ₆ (mg/day)						
<1.58	1.4	0.9–2.2	0.18	1.2	0.7–1.8	0.57
1.59–2.25	1.4	0.9–2.1	0.13	1.0	0.7–1.7	0.66
2.26–3.52	1.4	0.9–2.1	0.16	1.3	0.9–2.0	0.23
≥ 3.53	1.0			1.0		
			0.14‡			0.70‡

*Adjusted for sex, age, total:HDL cholesterol ratio, smoking status, systolic blood pressure, and energy intake.

†CI denotes confidence interval.

‡For trend.

concentrations and carotid-artery stenosis of ≥ 25 percent is independent of known risk factors for carotid-artery stenosis in the study cohort. The relation between traditional risk factors for cardiovascular disease and carotid-artery stenosis in these elderly members of the Framingham Heart Study cohort has been described previously.^{9,37} Age, smoking status, systolic blood pressure, and plasma cholesterol concentrations were significantly associated with the degree of carotid-artery stenosis in both sexes when examined prospectively,³⁷ and concurrently measured plasma HDL cholesterol was associated with the degree of stenosis in women only.⁹ The cholesterol concentration was not associated with concurrently determined carotid-artery stenosis.⁹ These findings are generally consistent with those of studies of other population-based cohorts of elderly people^{10,11} or nursing home residents.¹³

In our study, the risk of stenosis of ≥ 25 percent was increased in subjects with homocysteine concentrations previously believed to be normal on the basis of measurements in healthy populations. Stampfer et al.⁴ defined an elevated homocysteine concentration as one that exceeds 15.8 μmol per liter (95th percentile for healthy control subjects). Joosten et al.³⁸ defined an elevated homocysteine concentration as one that exceeded 13.9 μmol per liter (the mean value plus 2 SD among healthy young controls). In the Framingham Heart Study cohort, we had previously considered a homocysteine concentration of 14 μmol per liter to be elevated (90th percentile for persons with apparently adequate concentrations of folate, vitamin B₁₂, and vitamin B₆).²⁵ In the present study we found that the risk of stenosis was elevated in subjects with homocysteine concentrations between 11.4 and 14.3 μmol per liter. These data will require us to reconsider

the current definitions of elevated homocysteine concentrations.

We also examined the relations between specific nutritional determinants of hyperhomocysteinemia and stenosis in this elderly cohort. We have previously demonstrated the importance of plasma folate, vitamin B₁₂, and pyridoxal-5'-phosphate concentrations, as well as folate and vitamin B₆ intakes, to plasma homocysteine concentrations in this cohort.²⁵ Approximately two thirds of all cases of elevated homocysteine concentrations were associated with inadequate concentrations of one or more of these vitamins. In the present study we further demonstrated that plasma folate and pyridoxal-5'-phosphate concentrations and folate intake were linked to stenosis, in large part because of their regulation of plasma homocysteine concentrations (as indicated by the diminished odds ratios for stenosis after adjustment for homocysteine concentrations). Although there was some residual association between plasma folate concentrations and stenosis after adjustment for homocysteine concentrations, the likelihood-ratio test statistic suggests that the addition of the plasma folate concentration to a model containing the homocysteine concentration made no significant contribution. Measurement error and biologic variability in both folate and homocysteine might explain the residual folate association.

We have previously demonstrated that the majority of persons with elevated plasma homocysteine concentrations have insufficient concentrations of folate, vitamin B₁₂, or vitamin B₆, and others have demonstrated that innocuous regimens of vitamin supplementation (including folate, vitamin B₁₂, and vitamin B₆) effectively lower moderately elevated plasma homocysteine concentrations to the normal range.^{39,40} The results of our present study provide the rationale for a randomized, controlled trial of the effect of homocysteine-lowering vitamin therapy on morbidity and mortality from vascular disease in elderly people with hyperhomocysteinemia.

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